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14. ABSTRACT:

Malaria is the primary infectious disease threat facing the U.S. solider, and is the leading cause of all casualties during tropical deployments. The long-term objective of this project is to identify and prepare the malaria parasite forms causing severe anemia, and then apply functional genomics and bioinformatics tools to identify 15 to 30 proteins that could form the basis for an effective vaccine at both the pre-erythrocytic and blood stages of malaria infection. The project will then evaluate these lead candidates for their recognition by sera collected from immune individuals, in order to identify the leading 3 to 5 candidates for a blood stage vaccine that prevents severe malarial anemia.

15. SUBJECT TERMS
Severe Malaria, *P. Falciparum*, Microarrays, Proteomics, Vaccines

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INTRODUCTION:

Malaria is the primary infectious disease threat facing the U.S. solider, and is the leading cause of all casualties during tropical deployments. The long-term objective of this project is to identify and prepare the malaria parasite forms causing severe anemia, and then apply functional genomics and bioinformatics tools to identify 15 to 30 proteins that could form the basis for an effective vaccine at both the pre-erythrocytic and blood stages of malaria infection. The project will then evaluate these lead candidates for their recognition by sera collected from immune individuals, in order to identify the leading 3 to 5 candidates for a blood stage vaccine that prevents severe malarial anemia.

BODY:

The bulk of activity during this first year has been spent obtaining human subjects protocol approvals and optimizing assays related to our laboratory studies.

Protocol Review

Owing to the DOD requirement for a distinct protocol related to project activities, we prepared a document for submission to both our local IRB (Western IRB in Olympia, WA) and the HSRRB. An unofficial DOD review was conducted in November 2005 prior to us submitting the protocol to our local IRB. The protocol was then submitted to WIRB in January 2005, with subsequent approval that month. The locally IRB approved protocol was submitted to DOD for review in March.

Three Memorandums for the Record were issued with requests for information/clarification during the following months. The protocol was subsequently revised and DOD issued approval in December 2005. The protocol was then re-submitted to WIRB, with an approval issued on February 7, 2006. These documents were sent to DOD, who issued final approval on February 10, 2006.

RNA Stabilization and Extraction Assays

Our current method of RNA stabilization and extraction from Tanzanian whole blood clinical samples does not take steps to remove globin mRNA, which is produced by reticulocytes. Globin mRNA contamination in RNA extraction from whole blood can seriously reduce sensitivity and increase variability of DNA-microarrays data because globin mRNA levels vary widely patient-to-patient and can in cases exceed >70% of total labeled aRNA during hybridization. A process has been developed to remove globin contamination and is currently being optimized. Once optimized, the process will be implemented to improve DNA-microarray data quality in subsequent studies. In addition, studies are being done to streamline the RNA extraction process and reduce variability during amplification. If preliminary data is confirmed, the RNA extraction time will be shorted from 12 hours to 90 minutes.

Since it is difficult to know *a priori* the total number of patients samples which will be necessary to resolve transcriptional differences between malaria patients with severe anemia and malaria patients with normal hemoglobin levels, developing a relevant reference mRNA pool is important so that all samples can be quickly and accurately compared to each other. We are currently running studies to validate a reference mRNA pool. If the validation is successful, a reference-design microarray protocol will be implemented.

We are also in the process of accumulating and reviewing patient data in order to select suitable samples on which transcriptional analysis can be carried out to complete the severe anemia study.

Pilot Microarray Studies

Pilot studies have been completed using parasites from children with malarial anemia versus parasites from infected children without anemia using microarrays. To perform these pilot studies, we prepared RNA from four parasite samples in each group. Then performed labeling, cDNA synthesis, Cy3 Cy5 labeling and hybridization on oligonucleotide spotted arrays that measure *p. falciparum* gene expression levels. Each malaria anemia sample was compared to all non-anemia samples, for a 4 by 4 study design, with dye-swaps to exclude dye bias.

These pilot studies suggest that several genes are upregulated in the anemia samples, suggesting differential parasite gene expression related to disease outcomes. We selected a limited number of differentially expressed genes (by mircorarray analysis) to confirm by qPCR studies. For the three genes tested (PFL0030c, PFE1640w, chr12.gen_527), the qPCR results confirm the pattern observed by microarrays, suggesting that the arrays are yielding robust data. Of particular interest, the upregulated gene PFL0030c from anemia parasites has also been shown to be upregulated in parasites causing placenta malaria syndromes. For this reason, we are speculating that PFL0030c may be a common virulence marker of disease-causing malaria parasites.

The microarray studies are being expanded to a larger number of parasite samples collected from children. We currently have several dozen parasite samples from infected children with associated clinical information that will be used to perform array analysis.

KEY RESEARCH ACCOMPLISHMENTS:

Below is a list of key research accomplishments emanating from this research:

- Human Subjects Protocol Approvals from both local IRB and HSRRB
- Optimization of RNA stabilization and extraction assays
- Pilot microarray studies completed

REPORTABLE OUTCOMES:

15 November 2005, Oral presentation at the 4th MIM Pan-African Malaria Meeting: Using the parasite genome to solve the malaria problem. PE Duffy.

CONCLUSION:

Our pilot studies demonstrate the feasibility of performing mircroarray and proteomics studies on parasite samples collected from African infants and young children. The initial microarray results confirmed by qPCR studies suggest that specific parasite genes are differentially upregulated in samples causing malarial anemia. These results support our primary hypothesis for the proposed studies, however, they need to be confirmed in a much larger number of samples. If specific parasite genes and proteins are upregluated in the parasite forms causing severe malaria, we will want to assess these as targets of protective immune responses as well as candidates for severe malaria vaccines as described in our original DOD proposal.

REFERENCES:

None.

APPENDICES:

None.

SUPPORTING DATA:

None.